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In-vitro activity of 21 antimicrobial agents against Neisseria gonorrhoeae in Brussels

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SUMMARY The minimum inhibitory concentrations (MIC) of 21 antimicrobial agents was measured for 80 strains of *Neisseria gonorrhoeae* isolated in Brussels in 1978. Bimodal distributions were found for penicillin G, ampicillin, amoxycillin, carbenicillin, and cephalexin. Of the strains, 17·5% were relatively resistant to penicillin G (MIC >0·08 μg/ml), 27·5% to ampicillin (MIC >0·16 μg/ml), 23·8% to amoxycillin, and 43·3% to carbenicillin. Cefotaxime was the most active antibiotic, with MICs in the nanogram range; 3·8% and 5% of the strains were relatively resistant to cephaloridine and cephalexin respectively, but no strains were resistant to cefazolin, cefuroxime, or cefotaxime. Resistance to tetracycline, doxycycline, minocycline, erythromycin, and spiramycin (MIC >1 μg/ml) was found in 6·3%, 2·5%, 2·5%, 5%, and 51·3% of the strains respectively.

A very good correlation was present between chloramphenicol and thiamphenicol, with 16·3% and 10% of relatively resistant strains respectively. Only two isolates showed an MIC >1·25 μg/ml for rifampicin, and 10% of the strains needed ⩾12μg/ml of spectinomycin for complete inhibition of growth. A very high synergy was found for the 20 : 1 combination of sulphamethoxazole and trimethoprim, with only one isolate resistant to this combination. None of the strains tested produced β-lactamase.

Introduction

Many studies have been carried out in Western Europe over the last 20 years to test the in-vitro sensitivity of *Neisseria gonorrhoeae* to various antimicrobial agents. A definite progression towards decreased sensitivity to various antibiotics has been noticed.[1] Greater importance has been attached to the regional differences in the sensitivity patterns of gonococci.[2 3] It seems important, therefore, to continue screening the sensitivity of gonococci in different parts of the world. This paper describes quantitative sensitivity determinations of 80 unselected strains of *N gonorrhoeae* isolated before treatment in the venereal disease clinic at the St Pieters Hospital in Brussels from 1 January to 31 December 1978. The results are compared with those of other workers in other regions and with those of a similar study in the same hospital in 1976.[4]

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Materials and methods

ISOLATION OF GONOCOCCAL STRAINS

Eighty strains of *N gonorrhoeae* were isolated from male and female patients attending the venereal diseases clinic at the St Pieters Hospital in Brussels during 1978. Immediate diagnosis was made by the examination of Gram-stained smears. The strains were cultured on selective Thayer-Martin medium and incubated at 37°C in 10% CO_2 for 48 hours. The organisms were identified by colonial morphology, Gram-staining, oxidase activity, and sugar fermentation. The purified cultures were suspended in sterile horse serum and stored in liquid nitrogen until investigated further.

SENSITIVITY TESTING

Antibiotic sensitivity testing was performed by a dilution method in liquid medium. The strains were cultured on a medium containing brain-heart infusion (BHI) broth (Difco 0037-01) and 1·5% agar enriched with 10% defibrinated horse blood (Institut Pasteur, Brussels) and 1% sterile GC supplement (Oxoid SR 56). The plates were incubated in 10%

CO_2 for 48 hours. The resulting colonies were then suspended in a Mueller-Hinton broth to give 3×10^8 colony-forming units (cfu)/ml (McFarland No 1).

The medium used to test the sensitivity of the antimicrobial agents was Mueller-Hinton broth to which was added 1% Polyvitex (Bio-Merieux 5-5651), 5% of a haemin solution (600 μg/ml) (BDH-Biochemicals 24011), and 2% sterile horse serum. To test the sensitivity to the sulphonamides, 5% haemolysed horse blood (Institut Pasteur, Brussels) was used instead of haemin in the medium. The medium was dispensed in microtitre plates (Dynatech MA1501/N), together with two-fold dilutions of the antibiotics, by a Dynatech 96 Channel Dispenser. The test plates were inoculated with the suspension by a MIC 2000 Dynatech Inoculator which places 1 μl of the suspension in every test cup of 100 μl, giving a final concentration of 3×10^6 cfu/ml. In every test plate, one row was left without any antibiotic. Two reference strains of *N gonorrhoeae* (Reyn 181, Reyn 183) with known sensitivity patterns were tested simultaneously in each run. The plates were incubated in 10% CO_2 and examined after 48 hours in a Dynatech Viewbox. The lowest antibiotic concentration that inhibited bacterial growth completely, or almost completely, was regarded as the minimum inhibitory concentration (MIC).

ANTIMICROBIAL AGENTS

The following antimicrobial agents were tested: penicillin G (Continental Pharma), ampicillin (Bristol), amoxycillin and carbenicillin (Beecham Laboratories), cephaloridine, cephalexin, and cefazolin (Eli Lilly Laboratories), cefuroxime (Glaxo), cefotaxime (Hoechst), erythromycin (Abbott Laboratories), spiramycin (Specia), tetracycline HCL (Certa), doxycycline (Pfizer), minocycline (Lederle), chloramphenicol and rifampicin (Lepetit), thiamphenicol (Zambon), spectinomycin (Upjohn), trimethoprim and sulphamethoxazole (Roche SA) and a combination of sulphamethoxazole and trimethoprim in a 20 : 1 ratio.

Screening for β-lactamase production was performed by the chromogenic cephalosporin test (Glaxo compound 87/312).

Results

The distribution of the MICs for the 21 antimicrobial agents tested is shown in tables I and II. Bimodal distributions were found with penicillin G, ampicillin, amoxycillin, carbenicillin, and cephalexin (figure). Tests for β-lactamase production gave negative results for all the 80 strains.

Penicillin G showed a first peak at 0·048 μg/ml and a second at 0·012 μg/ml. We defined relative resistance (RR) as an MIC >0·08 μg/ml; 17·5% of the strains fell into this group, and 6·3% of the strains showed "high level resistance" to penicillin G (MIC >0·39 μg/ml). For ampicillin the two peaks occurred at 0·097 and 0·012-0·006 μg/ml and 27·5% of strains were considered to be relatively resistant (MIC >0·16 μg/ml).

The peaks for amoxycillin were at 0·195-0·097 μg/ml and 0·012-0·006 μg/ml; 23·8% of the strains were RR. Carbenicillin showed peaks at 0·195 μg/ml and 0·006 μg/ml; 43·3% were RR. A bimodal distribution was also found for cephalexin, with a first modus at 0·195-0·39 μg/ml and a second at 1·56 μg/ml. For the second modus strains of cephalexin only five out of 23 were also RR to penicillin G, while 10 strains were RR to ampicillin, nine to amoxycillin, and 15 to carbenicillin.

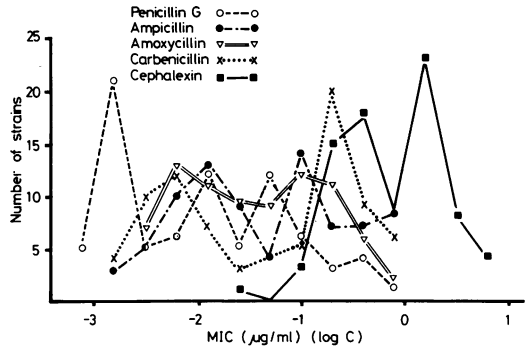

FIGURE *Distribution of MICs for penicillin G, ampicillin, amoxycillin, carbenicillin, and cephalexin.*

For the cephalosporins 3·8% of the strains were RR to cephaloridine (MIC >3·12 μg/ml), while 5% were RR to cephalexin. All were sensitive to cefazolin, cefuroxime, and cefotaxime. Among the tetracyclines, doxycycline and minocycline showed a comparable activity against the isolates. For tetracycline, doxycycline, and minocycline respectively 6·25, 2·5, and 2·5% of the isolates were RR (MIC >1μg/ml). Two isolates that were RR to tetracycline were also RR to penicillin G, while among the 14 strains that were RR to penicillin G only two were also RR to tetracycline.

Both chloramphenicol and thiamphenicol showed a peak at 0·195 μg/ml; 16·3% of the strains were RR to chloramphenicol and 10% to thiamphenicol. Erythromycin was more active than spiramycin, with only 5% being RR to it, while 51·3% were RR to spiramycin. For rifampicin two strains required 50 μg/ml or more to inhibit growth, but all the others (97·5%) were sensitive.

TABLE I *Distribution of MICs of 20 antimicrobial agents against 80 strains of gonococci*

	Minimum inhibitory concentrations (μg/ml)																		
Antimicrobial agents	*0·00037*	*0·00075*	*0·0015*	*0·003*	*0·006*	*0·012*	*0·024*	*0·048*	*0·097*	*0·195*	*0·39*	*0·78*	*1·56*	*3·12*	*6·25*	*12·5*	*25*	*50*	*100*	>100
Penicillin G		5	21	5	6	12	5	12	6	3	4	1								
Ampicillin			3	5	10	13	9	4	14	7	7	8								
Amoxycillin				7	13	11	9	9	12	11	6	2								
Carbenicillin			4	10	12	7	3	4	5	20	9	6								
Cephaloridine								4	6	10	17	25	11	4	1	2				
Cephalexin							1		3	15	18	8	23	8	4					
Cefazolin					4	2	3	8	15	13	22	6	7							
Cefuroxime		1	19	12	16	14	6	2	3	2	5									
Cefotaxime	28	22	18	9				1		1	1									
Erythromycin						1	6	4	20	23	13	9	2	2						
Spiramycin							1		3	6	6	23	16	16	5	3				
Tetracycline						3	11	8	14	17	11	11	3	2						
Doxycycline						5	13	14	18	15	8	5		1	1					
Minocycline					4	6	9	10	17	20	9	3	2							
Chloramphenicol							1	2	19	23	19	3	5	4	2	2				
Thiamphenicol							1		11	42	9	9	4	2	1	1				
Spectinomycin									2	4	2	5	7	25	27	5	2	1		
Rifampicin			1		12	8	19	21	12	4		1						1		1

	0·24	*0·48*	*0·97*	*1·95*	*3·9*	*7·8*	*15·6*	*31·2*	*62·5*	*125*	*250*
Sulfamethoxazole		2	4	16	19	26	8	3	2		

	0·024	*0·048*	*0·097*	*0·195*	*0·39*	*0·78*	*1·56*	*3·12*	*6·25*	*12·5*	*25*	*50*	*100*	>100
Trimethoprim						1	1		4	10	23	19	21	1

TABLE II *Minimum inhibitory concentration of 20 : 1 combination of sulphamethoxazole and trimethoprim*

	Sulphamethoxazole (μg/ml)								
Trimethoprim (μ/ml)	*0·12*	*0·24*	*0·48*	*0·97*	*1·95*	*3·9*	*7·8*	*15·6*	*31·2*
0·006	1								
0·012		1							
0·024			1						
0·048				3					
0·097					17				
0·195						31			
0·39							22		
0·78								3	
1·56									1

Ten per cent of the strains needed 12 μg/ml of spectinomycin for complete inhibition of growth. With the sulphonamides 51·3% were RR to trimethoprim (MIC >25 μg/ml) and 6·3% to sulphamethoxazole (MIC >15·6 μg/ml). The 20 : 1 combination of sulphamethoxazole and trimethoprim resulted in a significant reduction in the MICs.

The number of strains that were cross-resistant with penicillin G are shown in table III. We found no multiresistant strains. The contingency coefficient C, which measures the extent of correlation between two sets of attributes and is computed from 2 × 2 contingency table for all pairs of antimicrobial agents, is given in table IV. The significance of the C factor is tested by the χ^2 test.[5a]

A very good correlation was found between the different penicillins and the cephalosporins. Only penicillin G and ampicillin showed a correlation with cephalexin. The penicillins also had a poor correlation with the tetracyclines, except for amoxycillin, which showed a slightly better correlation with the tetracyclines. No correlation was found between the different cephalosporins, except for a slightly better correlation between cephalexin and cephaloridine.

TABLE III *Strains relatively resistant to penicillin G which showed cross-resistance to other antibacterial agents**

	Strains	
Antimicrobial agents	*No*	*%*
Ampicillin	9	64·3
Amoxycillin	8	57·1
Carbenicillin	11	78·6
Cephaloridine	1	7·1
Cephalexin	3	21·4
Cefazolin	0	0
Cefuroxime	0	0
Cefotaxime	0	0
Erythromycin	4	28·6
Spiramycin	13	92·9
Tetracycline	2	14·3
Doxycycline	1	7·1
Minocycline	0	0
Chloramphenicol	6	42·9
Thiamphenicol	4	28·6
Spectinomycin	5	35·7
Rifampicin	0	0
Trimethoprim	5	35·7
Sulphamethoxazole	1	7·1
Co-trimoxazole	0	0

*14 strains relatively resistant to penicillin G (MIC >0·08 μg/ml)

Erythromycin showed a poor correlation with spiramycin, and no correlation with the different tetracyclines. Tetracycline showed a good correlation with doxycycline and minocycline, but there was no correlation between the two latter antibiotics. The correlation between chloramphenicol and thiamphenicol was excellent. Rifampicin showed no correlation with any other antibiotic. This was also found for sulphamethoxazole; a correlation was only found between co-trimoxazole and sulphamethoxazole but not between the sulphonamides and any other of the antimicrobial agents tested.

Discussion

The results for penicillin G (17·5% RR) show that there has been a marked decrease in the proportion of RR strains of *N gonorrhoeae* since the study of Vanhoof *et al,*[4] in which 51% of the strains were RR. Meheus *et al*[5b] found a similar result as in the present study. A decrease in RR strains has already been described by Olsen and Lomholt[6] in Greenland and by Seth and Wilkinson[7] in London. For ampicillin, we found that 27·5% of the strains were RR. This is a lower percentage than that reported by Meheus *et al,*[5b] and much lower than in the former study of Vanhoof *et al* in Brussels.[4]

The other penicillins (amoxycillin and carbenicillin) also showed a lower proportion of RR strains than reported in the former Belgian studies. For cephalexin, a bimodal distribution was obvious, but only 17% of the second modus strains were also RR to penicillin G in contrast to the former study of Vanhoof *et al,*[4] in which all second modus strains of cephalexin were found to be RR to penicillin G.

In general, very good sensitivity was found for the new cephalosporins, with no RR strains at all and all MICs >0·78 μg/ml. Cefotaxime was the most active of all the antibiotics tested in this study. We found that 6·3% of the strains were RR to tetracycline,

TABLE IV *Contingency coefficient C computed from a 2 × 2 contingency table for all pairs of antimicrobial agents (significance by χ^2 test)*

	PEN	*AMP*	*AMX*	*CAR*	*CRD*	*CLX*	*CZL*	*CFX*	*CTX*	*ERY*	*SPI*	*TET*	*DOX*	*MIN*	*CHL*	*THI*	*SPE*	*RIF*	*TMP*	*SMZ*	*SXT*
PEN																					
AMP	0·47‡																				
AMX	0·34†	0·41‡																			
CAR	0·30†	0·35†	0·46†																		
CRD	0·08§	0·12§	0·20§	0·09§																	
CLX	0·33†	0·24*	0·14§	0·14§	0·25*																
CZL	0·00§	0·00§	0·00§	0·00§	0·00§	0·00§															
CFX	0·00§	0·00§	0·00§	0·00§	0·00§	0·00§	0·00§														
CTX	0·00§	0·00§	0·00§	0·00§	0·00§	0·00§	0·00§	0·00§													
ERY	0·32†	0·11§	0·01§	0·25*	0·05§	0·20§	0·00§	0·00§	0·00§												
SPI	0·36‡	0·02§	0·19§	0·15§	0·06§	0·22*	0·00§	0·00§	0·00§	0·22*											
TET	0·15§	0·18§	0·32†	0·19§	0·21§	0·06§	0·00§	0·00§	0·00§	0·18§	0·25*										
DOX	0·14§	0·10§	0·28*	0·18§	0·20§	0·10§	0·00§	0·00§	0·00§	0·04§	0·15§	0·29†									
MIN	0·07§	0·08§	0·28*	0·18§	0·20§	0·10§	0·00§	0·00§	0·00§	0·04§	0·15§	0·29†	0·04§								
CHL	0·32†	0·11§	0·07§	0·28†	0·24*	0·34*	0·00§	0·00§	0·00§	0·34†	0·22*	0·16§	0·34†	0·34†							
THI	0·27*	0·26*	0·11§	0·13§	0·35‡	0·45‡	0·00§	0·00§	0·00§	0·29†	0·16§	0·09§	0·21§	0·21§	0·60‡						
SPE	0·37‡	0·26*	0·11§	0·13§	0·15§	0·29†	0·00§	0·00§	0·00§	0·29†	0·08§	0·09§	0·21§	0·03§	0·39‡	0·40‡					
RIF	0·07§	0·10§	0·09§	0·02§	0·03§	0·04§	0·00§	0·00§	0·00§	0·04§	0·16§	0·04§	0·03§	0·03§	0·07§	0·05§	0·05§				
TMP	0·14§	0·13§	0·10§	0·15§	0·04*	0·12§	0·00§	0·00§	0·00§	0·12§	0·00§	0·06§	0·00§	0·00§	0·07§	0·17§	0·01§	0·16§			
SMZ	0·02§	0·04§	0·01§	0·08§	0·05§	0·18§	0·00§	0·00§	0·00§	0·06§	0·06§	0·07§	0·04§	0·04§	0·03§	0·09§	0·09§	0·04§	0·16§		
SXT	0·05§	0·18§	0·06§	0·13§	0·02§	0·03§	0·00§	0·00§	0·00§	0·03§	0·11§	0·03§	0·02§	0·02§	0·05§	0·04§	0·04§	0·02§	0·11§	0·40‡	

* $6·635 > \chi^2 > 3·841$, $0·01 < P < 0·05$
† $10·832 > \chi^2 > 6·635$, $0·001 < P < 0·01$
‡ $\chi^2 > 10·832$, $P < 0·001$
§ $3·841 > \chi^2$, $0·05 < P$

PEN = penicillin G; AMP = ampicillin; AMX = amoxycillin; CAR = carbenicillin; CRD = cephaloridine; CLX = cephalexin; CZL = cefazolin; CFX = cefuroxime; CTX = cefotaxime; ERY = erythromycin; SPI = spiramycin; TET = tetracycline; DOX = doxycycline; MIN = minocycline; CHL = chloramphenicol; THI = thiamphenicol; SPE = spectinomycin; RIF = rifampicin; TMP = trimethoprim; SMZ = sulphamethoxazole; SXT = co-trimoxazole.

which is very low compared with the studies of Stolz and Zwart,[2] Bergogne-Berezin,[1] Meheus *et al*,[5b] and Jaffe *et al.*[3] Doxycycline and minocycline showed comparable activity. Five per cent of the strains seemed to be RR to erythromycin, which is greater than that found by Meheus.[4] Robson and Salit,[8] and Givan and Keyl.[9] Erythromycin showed a much higher activity than spiramycin. Ten per cent of the strains needed $\geqslant 12$ μg/ml of spectinomycin to be inhibited. This seems to be high in comparison with other studies. Only about 2·5% of the strains were RR to rifampicin, and this agrees well with former results. Once again, the synergy between sulphamethoxazole and trimethoprim was noticed. Only one strain showed relative resistance to the 20:1 combination.

We did not find any β-lactamase-producing gonococcal strains in our population; these strains are extremely rare in Belgium.[10]

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